

positioning said prepared heterogeneous DNA segment into an expression vector suitable for transfection of endothelial cells in-situ.

REMARKS

In this non-final Official Action, the Examiner has rejected previously pending claims 3, 8-9 and 11 respectively under 35 U.S.C. 101 as being directed to non-statutory subject matter. In addition, the Examiners have rejected previously pending claims 1-2, 4-7, 9, 14-17 and 19 respectively under 35 U.S.C. 112, second paragraph, as being indefinite in language for specifically stated reasons. Finally, the Examiners have twice rejected the previously pending claims under 35 U.S.C. 102(b) as being anticipated individually by either the Saunders *et al.* patent [U.S. Patent No. 5,486,599] or the Stanley *et al.* 1995 publication [*J. Biol. Chem.* 270(10):5077-5083 (1995)].

In response, applicants have amended claims 1-7 and 12-13 respectively; cancelled without prejudice claims 9, 10, 14-17 and 19 respectively; and retained previously pending claims 8 and 11 respectively in unaltered form. By these amendments, cancellations, and the discussion presented hereinafter, applicants believe they have overcome and obviated each basis for rejection stated by the Examiner in the instant first Official Action.

As a preliminary matter, applicants wish to address the Examiners' stated request for clarification of the Declaration of record as concerns the spelling of co-inventor Volk's first name [as stated at page 5 of the Official Action]. It will be recognized and appreciated that this particular co-inventor is a German citizen; that his name is Germanic in origin; and that in proper German language form his first name is spelled with an "umlaut" – i.e., a mark (¨) used over a vowel in Germanic languages to indicate a vowel change. However, in a personal attempt to conform to the American style of the English language, Dr. Volk himself often, but not always, either chooses to inset the letter "e" into the spelling of his first name in place of the traditional German "umlaut"; or alternatively, chooses merely to omit the German umlaut entirely from his name. For this reason, his written name typically will appear in the alternative as "Ruediger" as well as "Rudiger" (but without the umlaut over the letter "u"). In addition, Dr. Volk, for his own personal reasons, chooses to use both alternative English language forms of his first name indiscriminately and at will. Accordingly, rather than now attempt to change Dr. Volk's personal habits regarding the current alternative spellings of his own first name in the Declaration or in any other document, applicants undersigned attorney earnestly requests that the Examiner be cognizant of the personal use circumstances and accept both first name spelling forms in the alternative as being correct for Dr. Volk.

Applicants will now address each of the different substantive bases for rejection stated by the Examiner in the instant Official Action with regard both to its legal requirements and its relevant factual circumstances. Yet, since so much of the Examiner's stated views and positions are dependent upon having a focused and clear understanding of what applicants' invention truly is - as defined by the language of the now pending claims, applicants deem it both useful and appropriate to review summarily here the subject matter which is now presented as applicants' claimed invention.

#### I. Applicants' Invention As Presently Claimed

Applicants' inventive subject matter as a whole is broadly disclosed and is generally applicable in-situ [wherein the term "in-situ" includes "in-vivo", "ex-vivo", and "in-vitro" environments]; is amply and extensively described for multiple use instances by the Specification text; and is properly encompassed in scope and well defined by the language of original claims 1-19 respectively, as submitted September 2, 1998.

However, owing to the Restriction Requirement imposed October 28, 1999 and the Examiner's continuing insistence that all pending claims in this application must be directed to "in-vitro" environments alone - applicants, via their undersigned attorney, have therefore chosen to focus and limit the scope of the claims now pending in this application to only

those claims which define the various compositions of matter generally (without reference to the particulars of the use environment) and those claims which alone recite methods for making the compositions of matter as such.

Thus, the original claims directed to employing these compositions of matter in specific environments [original claims 8-9] or directed to methods for stimulating angiogenesis in-situ [original claims 14-19] have been cancelled (without prejudice) from this application; and the substance and scope of these now cancelled claims will now be pursued separately in a Divisional application to be filed concurrently with this Response. This legal process and Divisional filing appears to be the sole means now available to applicants by which the full scope of applicants' invention as disclosed by the Specification text as well as those claims defining in-situ methods of use and in-situ applications broadly [including "in-vivo", "ex-vivo", and "in-vitro" environments] can be obtained.

Accordingly, applicants' invention is presently presented as compositions of matter generally useful in any in-situ environment and as methods for making these compositions. The claims now pending thus include: a prepared heterogeneous DNA segment coding for recombinant proteoglycans [amended independent claim 1]; a constructed expression capable of effecting transfection of endothelial cell in-situ for subsequent expression of recombinant proteoglycans [amended independent claim 2];

an in-situ endothelial cell which expresses and positions a recombinant proteoglycan at the endothelial cell surface [amended independent claim 3]; A method for making a prepared heterogeneous DNA segment coding for recombinant proteoglycans [amended independent claim 12]; and a method for making a constructed expression vector capable of effecting transfection of endothelial cell in-situ such that an overexpression of recombinant proteoglycans occurs [amended independent claim 13].

The Examiner will also realize that all the essential aspects of the invention defined by these presently pending amended independent claims are broadly disclosed, are described in detail, and are factually supported by the Specification text. Unfortunately however, each of these amended independent claims now pending recites merely some aspects and particular parts of applicants' inventive subject matter as a whole.

## II. The Rejection Of Some Claims Under 35 U.S.C. 101

The Examiner has rejected previously pending claims 3, 8-9 and 11 under 35 U.S.C. 101 because the claimed invention is allegedly directed to non-statutory subject matter. Specifically, the Examiner believes that scope for the these claims might encompass human beings, which are clearly non-statutory subject matter.

In response, applicants have cancelled claims 14-17 and 19, without prejudice; and amended the language of independent claim 3 to

define the inventive subject matter as " An in-situ transfected endothelial cell which comprises a living tissue...". In this manner, the scope of amended independent claim 3 (and of dependent claims 8 and 11) is stated in precisely recited terms as an in-situ transfected cell to be found as part of a living tissue. Thus, by the explicit language in the preamble, the scope of claim 3 is controlled and limited to a in-situ cell environment within a living tissue. This explicit wording does not recite or identify a living human being as such as the recited invention of the claim.

Accordingly, the subject matter recited by amended independent claim 3 (as well as of dependent claims 8 and 11 respectively) is deemed to be statutorily proper and correct. For these reasons, applicants respectfully request that the Examiner reconsider his position and withdraw this ground of rejection against the presently pending claims.

### III. The Rejection Under 35 U.S.C. 112, 2<sup>nd</sup> Paragraph

The Examiner has rejected previously pending claims 1-2, 4-7, 9, 14-17 and 19 under 35 U.S.C. 112, 2<sup>nd</sup> paragraph as being vague and indefinite in language. The Examiner's position is based on a range of wording problems in different claims. In response, applicants have cancelled claims 9, 14-17 and 19 without prejudice; and amended all of claims 1-2 and 4-7 respectively now pending. Applicants will address and summarize each specific question of language for these claims.

As regards independent claims 1-2, applicants have accepted the Examiner's offered suggestion and amended the language of these independent claims to recite -- a -- syndecan-4 molecule.

As regards dependent claim 9 and the vague metes and bounds of the claimed subject matter, applicants have herein cancelled claim 9 (without prejudice) in its entirety.

As regards claims 14-17 and 19 and the Examiner's stated reasons for language vagueness, applicants have herein cancelled each of claims 14-17 and 19 (without prejudice) entirety.

Finally, as regards the language of the pending claims as a whole, applicants note that the essential inquiry and legal requirement is to determine whether the language of the presently pending claims do set out and circumscribe a particular area or subject matter with a reasonable degree of precision and particularity. It is here where the meaning of the words and language employed to define the invention is analyzed; not in a vacuum, but always with regard to the teachings of the prior art and within the particular description, use or context disclosed by the Specification as it is understood and interpreted by one possessing ordinary skill in the pertinent art [In re Angstadt, 190 U.S.P.Q. 214 (C.C.P.A. 1976)].

Applicants note that each of the terms used in presently pending claims 1-7 and 12-13 respectively is well understood; is not subject to

numerous definitions and interpretations; and that there is no discrepancy, no confusion, and no ambiguity with regard to the antecedent descriptive basis and support provided by the Specification text. Rather, the language of the presently pending claims as a whole read on subject matter which is completely disclosed and enabled by the Specification text. Moreover, each recited element of the pending claims is explicit and clearly stated; and employs wording which sets forth and circumscribes the particular subject matter area with the requisite reasonable degree of precision and particularity [In re Moore, 169 U.S.P.Q. 236 (C.C.P.A. 1971)].

For these reasons, applicants respectfully submit that each and every claim now pending satisfies the requirements of precision, clarity, and particularity required by the second paragraph of 35 U.S.C. 112. Accordingly, applicants respectfully request that the Examiner reconsider his stated position and withdraw this ground of rejection against the presently pending claims.

#### IV. The Rejection Under 35 U.S.C. 102(b)

The Examiner has twice rejected the previously pending claims under 35 U.S.C. 102(b) as being anticipated individually either by the Saunders *et al.* patent [U.S. Patent No. 5,486,599], or by the Stanley *et al.* 1995 publication [*J. Biol. Chem.* 270(10):5077-5083 (1995)].

As a matter of long established law, anticipation under 35 U.S.C. 102(b) requires that a reference must describe the claimed subject matter with sufficient clarity and detail to establish that the subject matter existed in the prior art, and that such existence would be recognized by persons of ordinary skill in the field of the invention [In re Spada, 15 U.S.P.Q.2d 1655 at 1657 (Fed. Cir. 1990)]. Each required element or essential component of the claimed composition and/or the claimed process must be described or embodied, directly or indirectly, within a single reference. Anticipation thus requires exact identity or effective duplication of applicant's claimed invention; and the single prior art reference of record must describe applicant's claimed invention sufficiently in detail such that a person of ordinary skill in that field has possession of the invention itself.

Also, in deciding the issue of anticipation, the Examiner must identify each requisite element as recited within the claims; determine their meaning in light of the Specification's descriptive text; and identify the existence and presence for each of the corresponding elements as being disclosed within the allegedly anticipating reference [Scripts Clinical and Research Foundation vs. Genentech Inc., 18 U.S.P.Q. 2d 1001 (Fed. Cir. 1991); Glaverbel Society Anonyme vs. Northlake Marketing and Supply Inc., 35 U.S.P.Q. 2d 1496 (Fed. Cir. 1995)].

It is also useful here to identify the proper legal basis and standard for determining obviousness under 35 U.S.C. 103. Where applicants' claimed subject matter can be rejected as obvious in view of a single reference (or a combination of prior art references), a proper analysis must consider inter alia two factors: (1) whether the prior art of record would have suggested to those of ordinary skill in the art that they should carry out the claimed process or make the claimed composition; and (2) whether the prior art would also have revealed that in so carrying out or making, those of ordinary skill would have a reasonable expectation of success [In re Dow Chemical Company, 5 U.S.P.Q. 2d 1529 (Fed. Cir. 1988)]. Both the suggestion and the reasonable expectation of success must be found directly within the text of the prior art reference(s) itself and cannot be derived or extrapolated from applicant's disclosure [In re Vaeck, 20 U.S.P.Q. 2d 1438 (Fed. Cir. 1991)]. In addition, the same inquiry must be carried out in the context of a purported "obvious modification" of the prior art information. The mere fact that the prior art might be modified in the manner suggested by an Examiner does not make that modification obvious unless the prior art suggested the desirability of the modification [In re Fritch, 23 U.S.P.Q. 2d 1780 (Fed. Cir. 1992) and the references cited therein].

Applicants therefore respectively submit that the Examiner's stated views and conclusions in the instant Official Action have failed to conform

to the legal standard and requirements for anticipation (as well as for obviousness). A summary review of the informational content within the each of the cited and applied prior art references will reveal the errors in the Examiner's stated position.

*A. The factual content of the Saunders et al. patent [U.S. Patent No. 5,486,599]:*

1. The Saunders *et al.* patent discloses the molecular cloning, sequencing and subsequent selective mutation of full length cDNAs encoding one, and only one, cell surface proteoglycan – **the syndecan-1 core protein** [Column 5, lines 55-67]. The text describes full length syndecan-1 cDNAs which are naturally existing genetic sequences derived and isolated from mouse mammary epithelial cells; and these syndecan-1 cDNAs encode a naturally occurring 311 amino acid core protein having several structural features consistent with its role as a proteoglycan cell surface receptor able to bind heparan sulfate [Column 8, lines 5-65].

2. The structure of the cDNA encoding and expressing the naturally existing syndecan-1 glycoprotein is described [Column 6, lines 49-67; SEQ. ID No. 1]. Using this naturally occurring cDNA, the Saunders *et al.* invention then intentionally generates specific mutations to one particular portion of the cDNA sequence to yield a point mutated syndecan-1 DNA and for subsequent expression of a genetically altered protein [see Column 8, line 5 through Column 16, line 7].

3. An analysis and review of the core protein structure for the naturally occurring syndecan-1 proteoglycan is described in detail [Column 16, line 8 through Column 18, line 33]. These details include a review of: the syndecan-1 protein characteristics [Column 16, lines 10-56]; the fine-structure aspects of the syndecan-1 protein core [Column 16, line 57-64; fig. 1]; the first hydrophobic stretch of the syndecan-1 protein [Column 16, lines 65-76 and Column 17, lines 1-9]; the second hydrophobic stretch of the syndecan-1 protein [Column 17, lines 10-18]; the position of the transmembrane domain for the syndecan-1 protein [Column 17, lines 19-32]; and the extracellular domain of the syndecan-1 protein [Column 17, lines 33-52].

4. The Saunders *et al.* Specification then describes the analysis of proteins produced from specified point mutations and truncated mutations in the ectodomain or extracellular domain of the naturally occurring syndecan-1 gene [Column 18, lines 16-33]. In addition, the Specification provides (via Fig. 2) an informational sequence analysis and alignment comparison of **the amino acid sequences surrounding the heparan sulfate attachment sequence of syndecan-1 protein is compared with the heparan sulfate attachment sequence of other syndecan homologs** designated as syndecan-2, syndecan-3 and syndecan-4 as well as for point mutations of syndecan-1 [Column 18, lines 2—25]. The informational comparison and analysis of Fig. 2 serves to

identify a consensus sequence which is required for attachment of heparan sulfate chains to **mutated forms of syndecan-1 DNA and expressed protein** [Column 18, lines 25-33].

5. The Saunders *et al.* disclosure then describes the preparation and subsequent expression of different recombinant syndecan-1 protein structures – all of which are encoded by mutant DNA forms expressly derived from the naturally occurring syndecan-1 cDNA [Column 21, lines 1-48]. All of these recombinant syndecan-1 forms must encode all or a part of the syndecan-1 DNA sequences containing the functional heparan sulfate attachment sequence which is positioned within the extracellular domain [see Columns 19 and 20]. Also, a preferred form of mutated syndecan-1 DNA is disclosed which can be produced with attached heparin sulfate chains attached when the mutant DNA sequence is functionally inserted into a vector that is subsequently expressed in a eukaryotic cell containing an enzyme system capable of producing heparan sulfate glycosaminoglycan chains [Column 21, lines 4-16].

6. The novelty and essence of the Saunders *et al.* invention thus is clearly limited to: a genetically altered extracellular domain sequence within the syndecan-1 cDNA, which is derived solely from the naturally occurring syndecan-1 DNA encoding the syndecan-1 core protein, but which has been prepared as a mutated cDNA sequence for the subsequent expression of a recombinant syndecan-1 core protein. This mutated

syndecan-1 DNA segment is positioned within the ectodomain or extracellular domain of the syndecan-1 DNA; and has been genetically modified to encode a protease susceptible sequence extracellularly adjacent the transmembrane region of the peptide as well as to provide a specified heparan sulfate attachment sequence for the subsequently expressed recombinant syndecan-1 protein. In particular, this ectodomain positioned specified mutated sequence must include at least one glycosylation site for the attachment of a heparin sulfate chain to said extracellular region [Claim 1; Abstract]; and the glycosylation site encoded by the mutant syndecan-1 DNA must provide a specified heparan sulfate attachment sequence for the expressed recombinant syndecan-1 core protein – a sequence represented by the formula Xac-Z-Ser-Gly-Ser-Gly, where Xac represents an amino acid residue having an acidic side chain, and where Z represents from 1-10 amino acid residues [Column 3, line 9 through Column 4, line 13]. In all other respects (including the DNA encoding the cytoplasmic domain and the transmembrane domain), the mutated DNA sequence codes for solely the naturally occurring syndecan-1 molecule.

This summary conveys the true facts and informational value of the extensive descriptive and technical content provided by the Saunders *et al.* patent reference.

*B. The factual content of the Stanley et al. 1995 publication [J. Biol. Chem. 270(10):5077-5083 (1995)].*

1. The Stanley *et al.* publication reports scientific research and empirical data demonstrating the ability to induce an aggregation of cells (isolated from the peripheral blood of a patient with plasma cell leukemia) following their transfection with a cDNA coding for a naturally occurring murine syndecan-1 protein [Page 5077, right column, 2<sup>nd</sup> full paragraph].

2. The Stanley *et al.* publication states that these patient derived cells (designated as ARH-77 cells) do not express syndecan-1 protein and have low levels of extractable heparan sulfate proteoglycan [Page 5077, right column, 3<sup>rd</sup> full paragraph]. However, after being transfected with a pMAMneo plasmid containing an inserted cDNA for murine syndecan-1 protein, the transfected cells are able to express syndecan-1 core protein on the cell surface and are able to form multicellular aggregates which are mediated by heparan sulfate [Pages 5078, right column; page 5079, left and right columns]. Also, the empirical data shows that the transfected cell aggregation is inhibited by: an introduction of exogenous syndecan-1 protein to the transfected cells; by the addition of heparin and heparin-like glycosaminoglycans to the transfected cells; and by the removal of heparan sulfate from the transfected cell surface [Pages 5079 and 5080; Abstract].

3. For empirical control and data comparison purposes only, Stanley *et al.* employed ARH-77 cells transfected with the pcDNA3 vector carrying the full coding region for rat syndecan-4, a naturally occurring DNA and expressed proteoglycan [Page 5077, right column, bottom]. The purpose expressly stated by Stanley *et al.* was "to determine if other syndecans could act to mediate cell aggregation" [Page 5081, left column, 1<sup>st</sup> full paragraph]. Thus, for empirical comparison purposes, these syndecan-4 transfected cells were found to behave similarly to cells transfected with syndecan-1; they formed large aggregates in culture and aggregated extensively in empirical assays [page 5081, Table II]. The syndecan-4 transfected cells also aggregated in a heparan sulfate-dependent manner.

4. Stanley *et al.* also explicitly state what is the true value and substance of their reported empirical data: their investigation provides the first direct evidence that syndecans participate in cell-to-cell adhesion [Page 5081, right column, bottom]. These authors also openly state their personal conclusions concerning their use of syndecan-4 transfected ARH-77 cells, in the following words: "The present studies indicate that both syndecan-1 and -4, which have very similar core proteins, bear functionally similar heparan sulfate chains in regard to their ability to mediate cell aggregation" [Page 5082. right column, last paragraph].

This summary conveys the true value and substantive content of the information and empirical data provided by the Stanley *et al.* publication.

*C. Some major differences and distinctions of applicants' claimed invention from each of the individually cited and applied references.*

(i). Both the Saunders *et al.* and the Stanley *et al.* references are concerned primarily, if not exclusively, with the DNA and the expressed protein of syndecan-1. The information and knowledge disclosed by each reference centers and is focused upon DNA encoding syndecan-1 and a characterization of the properties exhibited by an expressed syndecan-1 core protein structure.

(ii). The Saunders *et al.* invention creates and employs only derived and point mutated forms of syndecan-1 cDNA; and, after generating a mutated syndecan-1 DNA sequence, produces an expressed recombinant form of syndecan-1 core protein whose structure must present an altered extracellular domain having a specifically formulated glycosaminoglycan site for the binding of heparan sulfate within the domain. Moreover, there is no factual basis within the Saunders *et al.* reference for using or modifying any other DNA except syndecan-1 DNA in the manner described. Accordingly, the DNA coding for both the cytoplasmic domain and the transmembrane domain in the mutated DNA sequence and in the

subsequently expressed recombinant protein are always syndecan-1 molecules.

(iii). The Stanley *et al.* publication utilizes only the naturally occurring form of rat syndecan-1 protein and of rat syndecan-4 protein; and compares ARH-77 transfected cells expressing syndecan-1 protein to ARH-77 transfected cells expressing the naturally occurring form of rat syndecan-4 protein solely in order to assess the degree of cell aggregation demonstrably mediated by heparan sulfate. Thus, although utilized as a basis for empirical comparison, the ARH-77 cells transfected with the naturally occurring form of syndecan-4 DNA do not teach and can not suggest the possibility, much less the actual existence, of heterogeneous syndecan-4 DNA segments or of particular recombinant proteoglycan entities which are not a naturally occurring syndecan-4 molecule.

(iv). Applicants' invention as presently claimed will yield only a particular kind of recombinant syndecan-4 core protein. All the presently pending claims expressly recite the requirement that the DNA sequence and the expressed amino acid sequence for the recombinant proteoglycan is not a naturally occurring form of syndecan-4 molecule. Thus, by definition for each of the presently pending claims - while the cytoplasmic domain of the heterogeneous DNA segment and the expressed recombinant proteoglycan is always a syndecan-4 molecule, at least one sequence (DNA or amino acid) specifying the transmembrane domain

and/or the extracellular domain is not and can not be a naturally occurring syndecan-4 molecule. In this manner, the embodiments of applicants' invention as claimed are always directed to a recombinant form of syndecan-4 molecule, whether defined as a heterogeneous DNA segment and/or an expressed recombinant protein structure.

*D. The Examiner's conclusions are erroneous.*

Applicants also respectfully submit and maintain that there are many other substantive differences and particulars identified by the factual summaries stated above which separate and distinguish applicants' invention from each of the cited and applied references individually; that these multiple differences are substantive, major and distinctive; and that the information directly taught and/or indirectly implied by either the Saunders *et al.* patent or the Stanley *et al.* publication individually or collectively are radically remote from and irrelevant to applicants' invention as presently claimed.

In addition, applicants respectfully submit and maintain that none of the required elements or essential components recited by applicants' claimed compositions and/or applicants' claimed processes are described or embodied, directly or indirectly, within either the Saunders *et al.* patent or the Stanley *et al.* publication. Thus, neither reference provides the requisite exact identity or effective duplication of applicant's claimed

invention; and neither prior art reference of record describes applicant's claimed invention sufficiently or in similar detail such that a person of ordinary skill in this field could have possession of applicants' invention as claimed.

For all these reasons, applicants respectfully request that the Examiner reconsider his stated position and withdraw these grounds of rejection against the presently pending claims.

Applicants have addressed each basis of rejection stated in the instant Official Action forthrightly and objectively. In applicants' view, each issue or controversy has been evaluated, acted upon and resolved completely. For these reasons, applicants respectfully submit and affirm that presently pending claims 1-8 and 11-13 define patentable subject matter and are therefore now allowable.

In view of the above discussion and detailed review, applicants believe that this case is now in condition for allowance and reconsideration is respectfully requested. The Examiner is invited to call applicants' undersigned attorney should he feel that such a telephone call would further the prosecution of the present application.

Respectfully submitted,

SIMONS *et al.*

*Jan 27, 2003*

By: *David Prashker*

David Prashker

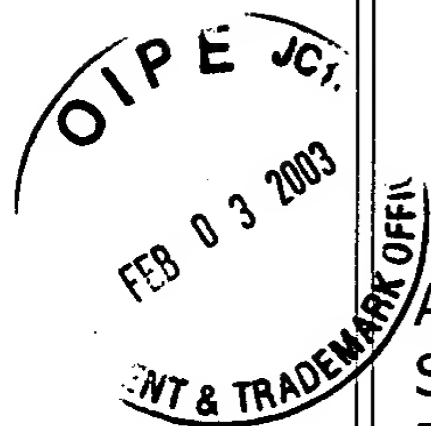
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Attachments  
# 24

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS : Simons et al.  
SERIAL NO. : 09/145,916  
FILED : September 2, 1998  
FOR : "STIMULATION OF ANGIOGENESIS VIA  
ENHANCED ENDOTHELIAL EXPRESSION  
OF SYNDECAN-4 CORE PROTEINS"  
EXAMINER : David Guzo  
GROUP ART UNIT : 1636  
ATTORNEY'S DOCKET NO. : BIS-039

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addressed to Assistant Commission for Patents, Washington, D.C.  
20231

on: Jan. 27, 2003

Attorney for applicants: David Prashker  
Signature: David Prashker  
Date: Jan. 27, 2003

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MARKED UP VERSION OF AMENDED CLAIMS  
SUBMITTED PURSUANT TO 37 C.F.R.1.121

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Applicants, in fulfillment of and in accordance with the requirements of 37 C.R.F. 121(c), hereby submit a marked up version of presently amended claims 1-7 and 12-13; cancelled claims 9, 10, 14-17, and 19; and unchanged pending claims 8 and 11 respectively, whose language is given below.

In addition, in view of the explicit holdings of the U.S. Supreme Court in the *Festo* case recently decided on May 28, 2002 [*Festo Corp. v. Shoketsu Kinzoku Kabushiki Co. Ltd. et al.*, 62 U.S.P.Q.2d 1705 (2002)] concerning the application of the legal doctrine of equivalents to amended claim language, applicants hereby present a formal attestation and affirmation of their legal position and rights: Applicants do not now surrender for any reason, nor have previously surrendered at any time or for any reason during the prosecution of the instant application, any inventive subject matter which is or could be expected to be a particular equivalent of the invention defined by the language of the amended claims then pending by a person ordinarily skilled in this art; and that no presumption of estoppel, either in law or equity, exists or pertains now or at any time previously as a potential bar to the application of the doctrine of equivalence for any and all possible embodiments which may be found to be encompassed now or in the future by the language of the amended claims proffered now or at any time previously for examination to the U.S. Patent Office. Accordingly,

applicants affirmatively rebut and explicitly dispute any presumption that the doctrine of equivalence for the language of the amended claims has been surrendered or is not in full force for any reason and at any time during the prosecution of any and all amended claims prosecuted for the instant application.

Presently amended claims 1-7 and 12-13; presently cancelled claims 9, 10, 14-17, and 19; and presently unchanged claims 8 and 11 respectively, whose language is now offered for review by the Patent Examiners of record, are as follows:

1 (Twice Amended). A prepared heterogeneous DNA segment for placement in [a suitable] an expression vector [and] capable of effecting transfection of endothelial cells in-situ such that overexpression of extracellular matrix heparan sulphate proteoglycans subsequently occurs in-situ, said prepared heterogeneous DNA segment comprising:

at least one first DNA sequence coding for the extracellular domain of a [discrete] recombinant proteoglycan entity that is not a naturally occurring form of syndecan-4 molecule, and which can be expressed by a transfected endothelial cell in-situ after being transfected with an expression vector carrying said first DNA sequence, said extracellular domain first DNA sequence specifying the extracellular N-terminal portion of [an] said expressed recombinant proteoglycan entity which [is] then [located at and]

extends from the endothelial cell surface and is capable of binding heparan sulfates to form an extracellular matrix in-situ;

at least one second DNA sequence coding for the transmembrane domain of [a discrete] said recombinant proteoglycan entity that is not a naturally occurring form of syndecan-4 molecule, and which can be expressed by a transfected endothelial cell in-situ after being transfected with an expression vector carrying said second DNA sequence, said transmembrane domain second DNA sequence specifying the medial portion of [an] said expressed recombinant proteoglycan entity which [is] then [located at and] extends through the endothelial cell membrane and is joined with said extracellular N-terminal portion of said expressed recombinant proteoglycan entity; and

at least one third DNA sequence coding for the cytoplasmic domain of [the] a syndecan-4 molecule in said [discrete] recombinant proteoglycan entity, and which can be [that is] expressed by a transfected endothelial cell in-situ after being transfected with an expression vector carrying said third DNA sequence, said syndecan-4 cytoplasmic domain third DNA sequence specifying the cytoplasmic portion of [an] said expressed recombinant proteoglycan entity which is then present within the cytoplasm of a transfected endothelial cell and is joined to said transmembrane portion [and said extracellular N-terminal portion] of said expressed recombinant proteoglycan entity.

2 (Once Amended). A constructed expression vector [for] capable of effecting transfection of endothelial cells in-situ such that overexpression of extracellular matrix heparan sulfate proteoglycan subsequently occurs in-situ, said constructed expression vector comprising:

a prepared heterogeneous DNA segment comprised of

(i) at least one first DNA sequence coding for the extracellular domain a [discrete] recombinant proteoglycan entity that is not a naturally occurring form of syndecan-4 molecule, and which can be expressed by a transfected endothelial cell in-situ after being transfected with an expression vector carrying said first DNA sequence, said extracellular domain first DNA sequence specifying the extracellular N-terminal portion of [an] said expressed recombinant proteoglycan entity which [is] then [located at and] extends from the endothelial cell surface and is capable of binding heparan sulfates to form an extracellular matrix in-situ,

(ii) at least one second DNA sequence coding for the transmembrane domain of [a discrete] said recombinant proteoglycan entity that is not a naturally occurring form of syndecan-4 molecule, and which can be expressed by a transfected endothelial cell in-situ after being transfected with an expression vector carrying said second DNA sequence, said transmembrane domain second DNA sequence specifying the medial portion of [an] said expressed recombinant proteoglycan entity which [is] then

[located at and] extends through the endothelial cell membrane and is joined with said extracellular N-terminal portion of said recombinant proteoglycan entity, and

(iii) at least one third DNA sequence coding for the cytoplasmic domain of [the] a syndecan-4 molecule in said [discrete] recombinant proteoglycan entity, and which can be [that is] expressed by a transfected endothelial cell in-situ after being transfected with an expression vector carrying said third DNA sequence, said syndecan-4 cytoplasmic domain third DNA sequence specifying the cytoplasmic portion of [an] said expressed recombinant proteoglycan entity [and] which is then present within the cytoplasm of a transfected endothelial cell and is joined to said transmembrane portion [and said extracellular N-terminal portion] of said expressed recombinant proteoglycan entity; and

an expression vector carrying said prepared heterogeneous DNA segment and [suitable for] capable of effecting transfection of endothelial cells in-situ.

3 (Twice Amended). An in-situ transfected endothelial cell which comprises a living tissue, overexpresses extracellular matrix heparan sulfate recombinant proteoglycans, and positions the recombinant proteoglycans at the endothelial cell surface, said in-situ transfected endothelial cell comprising:

a viable endothelial cell [previously] transfected in-situ with a constructed expression vector such that said transfected endothelial cell overexpresses [discrete] extracellular matrix heparan sulfate recombinant proteoglycan entities [coded for by said vector] that are not a naturally occurring form of syndecan-4 molecule, said overexpressed recombinant proteoglycan entities being comprised of

(i) an extracellular N-terminal portion for said recombinant proteoglycan entity that is not a naturally occurring form of syndecan-4 molecule and which [is located at and] extends from the transfected endothelial cell surface and [which] binds heparan sulfates to form an extracellular matrix in-situ, said extracellular N-terminal portion being the expressed product of at least one first DNA sequence [in] carried by the constructed expression vector and coding for the extracellular domain of said recombinant proteoglycan entity expressed by the transfected endothelial cell in-situ,

(ii) a transmembrane medial portion for said recombinant proteoglycan entity that is not a naturally occurring form of syndecan-4 molecule and which [is located at and] extends through the endothelial cell membrane and is joined with said extracellular N-terminal portion of said recombinant proteoglycan entity, said transmembrane medial portion being the expressed product of at least one second DNA sequence [in the prepared] carried by the constructed expression vector and coding for the

transmembrane domain of said recombinant proteoglycan entity expressed by the transfected endothelial cell in-situ, and

(iii) a syndecan-4 cytoplasmic portion for said recombinant proteoglycan entity which is present within the cytoplasm of the transfected endothelial cell [which] and is joined to said transmembrane portion [and said extracellular N-terminal portion] of said recombinant proteoglycan [entity], said syndecan-4 cytoplasmic portion being the expressed product of at least one third DNA sequence [in] carried by the constructed expression vector and coding for the cytoplasmic domain of the syndecan-4 molecule of said recombinant proteoglycan entity expressed by the transfected endothelial cell in-situ.

4 (Once Amended). The prepared heterogeneous DNA segment as recited by claim 1 wherein said first DNA sequence coding for the extracellular domain of [a discrete] said recombinant proteoglycan entity is selected from the group consisting of syndecan DNA sequences, glypican DNA sequences and perlecan DNA sequences.

5 (Once Amended). The prepared heterogeneous DNA segment as recited by claim 1 wherein said second DNA sequence coding for the transmembrane domain of [a discrete] said recombinant proteoglycan entity

is selected from the group consisting of syndecan DNA sequences, glypican DNA sequences and perlecan DNA sequences.

6 (Once Amended). The constructed expression vector as recited by claim 2 wherein said expression vector [suitable for] capable of effecting transfection of endothelial cells in-situ is a plasmid.

7 (Once Amended). The constructed expression vector as recited by claim 2 wherein said expression vector [suitable for] capable of effecting transfection of endothelial cells in-situ is a virus.

8 (Original). The in-situ transfected endothelial cell as recited by claim 3 wherein said cell is selected from the group consisting of vascular endothelial cells and dermal endothelial cells.

**Cancelled:** [9. The in-situ transfected endothelial cell as recited by claim 3 wherein said cell exists under in-vivo conditions.]

**Cancelled:** [10. The in-situ transfected endothelial cell as recited by claim 3 wherein said cell exists under in-vitro conditions.]

11 (Original). The in-situ transfected endothelial cell as recited by claim 3 wherein said transfected endothelial cell exists in a tissue comprising at least one kind of muscle cell selected from the group consisting of myocardial muscle cells, smooth muscle cells and striated muscle cells.

12 (Twice Amended). A method for making a prepared heterogeneous DNA segment intended for placement in [a suitable] an expression vector [and] capable of effecting transfection of endothelial cells in-situ such that overexpression of extracellular matrix heparan sulfate recombinant proteoglycan entities [subsequently] occurs in-situ, said method comprising the steps of:

obtaining at least one first DNA sequence coding for the extracellular domain of a [discrete] recombinant proteoglycan entity that is not a naturally occurring form of syndecan-4 molecule, and which can be expressed by a transfected endothelial cell in-situ after being transfected with an expression vector carrying said first DNA sequence, said extracellular domain first DNA sequence specifying the extracellular N-terminal portion of [an] said expressed recombinant proteoglycan entity which [is] then [located at and] extends from the transfected endothelial cell surface and is capable of binding heparan sulfates to form an extracellular matrix in-situ;

acquiring at least one second DNA sequence coding for the transmembrane domain of [a discrete] said recombinant proteoglycan entity

that is not a naturally occurring form of syndecan-4 molecule, and which can be expressed by a transfected endothelial cell in-situ after being transfected with an expression vector carrying said second DNA sequence, said transmembrane domain second DNA sequence specifying the medial portion of [an] said expressed recombinant proteoglycan entity which [is] then [located at and] extends through the transfected endothelial cell membrane and is joined with said extracellular N-terminal portion of said expressed recombinant proteoglycan entity;

procuring at least one third DNA sequence coding for the cytoplasmic domain of [the] a syndecan-4 molecule in said [discrete] recombinant proteoglycan entity, and which can be [that is] expressed by a transfected endothelial cell in-situ after being transfected with an expression vector carrying said third DNA sequence, said syndecan-4 cytoplasmic domain third DNA sequence specifying the cytoplasmic portion of [an] said expressed recombinant proteoglycan entity which is then present within the cytoplasm of an transfected endothelial cell and is joined to said transmembrane portion [and said extracellular N-terminal portion] of said expressed recombinant proteoglycan entity; and

joining together said extracellular domain first DNA sequence, said transmembrane domain second DNA sequence, and said syndecan-4 cytoplasmic domain third DNA sequence as a [discrete] prepared heterogeneous DNA segment.

13 (Twice Amended). A method for making a constructed expression vector [intended for] capable of effecting transfection of endothelial cells in-situ such that overexpression of extracellular matrix heparan sulfate recombinant proteoglycan entities [proteoglycans] subsequently occurs in-situ, said method comprising the steps of:

obtaining a prepared heterogeneous DNA segment comprised of

(i) at least one first DNA sequence coding for the extracellular domain of a [discrete] recombinant proteoglycan entity that is not a naturally occurring form of syndecan-4 molecule, and which can be expressed by a transfected endothelial cell in-situ after being transfected with an expression vector carrying said first DNA sequence, said extracellular domain first DNA sequence specifying the extracellular N-terminal portion of [an] said expressed recombinant proteoglycan entity which [is] then [located at and] extends from the transfected endothelial cell surface and is capable of binding heparan sulfates to form an extracellular matrix in-situ,

(ii) at least one second DNA sequence coding for the transmembrane domain of [a discrete] said recombinant proteoglycan entity that is not a naturally occurring form of syndecan-4 molecule, and which can be expressed by a transfected endothelial cell in-situ after being transfected with an expression vector carrying said second DNA sequence, said transmembrane domain second DNA sequence specifying the medial portion

of an expressed proteoglycan entity which [is] then [located at and] extends through the transfected endothelial cell membrane and is joined with said extracellular N-terminal portion of said expressed recombinant proteoglycan entity, and

(iii) at least one third DNA sequence coding for the cytoplasmic domain of the syndecan-4 molecule in [a discrete] said recombinant proteoglycan entity, and which can be [that is] expressed by a transfected endothelial cell in-situ after being transfected with an expression vector carrying said third DNA sequence, said syndecan-4 cytoplasmic domain third DNA sequence specifying the cytoplasmic portion of an expressed proteoglycan entity which is then present within the cytoplasm of said transfected endothelial cell and is joined to said transmembrane portion [and said extracellular N-terminal portion] of said expressed recombinant proteoglycan entity; and

positioning said prepared heterogeneous DNA segment into an expression vector suitable for transfection of endothelial cells in-situ.

**Cancelled:** [14. A method for stimulating angiogenesis in-situ within living tissues comprising vascular endothelial cells, said method comprising the steps of:

transfecting vascular endothelial cells in-situ with a constructed expression vector such that the resulting transfected vascular endothelial

cells overexpress discrete extracellular matrix heparan sulphate proteoglycan entities coded for by said constructed expression vector, said overexpressed proteoglycan entities being comprised of

(i) an extracellular N-terminal portion which is located at and extends from a transfected vascular endothelial cell surface and binds heparan sulphates to form an extracellular matrix in-situ, said extracellular N-terminal portion being the expressed product of at least one first DNA sequence in the constructed expression vector coding for the extracellular domain in a proteoglycan entity expressed by a transfected vascular endothelial cell in-situ after being transfected with said first DNA sequence,

(ii) a transmembrane medial portion which is located at and extends through a transfected vascular endothelial cell membrane and is joined with said extracellular N-terminal portion of said expressed proteoglycan entity, said transmembrane medial portion being the expressed product of at least one second DNA sequence in the constructed expression vector coding for the transmembrane domain of said proteoglycan entity expressed by a transfected vascular endothelial cell in-situ after being transfected with said second DNA sequence, and

(iii) a syndecan-4 cytoplasmic portion present within the cytoplasm of a transfected vascular endothelial cell which is joined to said transmembrane portion and said extracellular N-terminal portion of said expressed proteoglycan entity, said syndecan-4 cytoplasmic portion being

the expressed product of at least one third DNA sequence in the constructed expression vector coding for the cytoplasmic domain of the syndecan-4 molecule of said proteoglycan entity expressed by a transfected vascular endothelial cell in-situ after being transfected with said third DNA sequence; and

allowing said transfected vascular endothelial cells bearing said overexpressed extracellular matrix proteoglycan entities to stimulate angiogenesis in-situ.]

**Cancelled:** [15. The method for stimulating angiogenesis in-situ as recited by claim 14 wherein said living tissue comprises at least one other type of cell selected from the group consisting of muscle cells, fibrocytes and fibroblasts, epithelial cells, osteocysts and osteoblasts, erythrocytes and leukocytes, and neurons.]

**Cancelled:** [16. The method for stimulating angiogenesis in-situ as recited by claim 14 wherein said living tissue comprises at least one tissue selected from the group consisting of myocardium, lung, brain, kidney, spleen, liver, and gastro-intestinal tissues.]

**Cancelled:** [17. The method for stimulating angiogenesis in-situ as recited by claim 14 wherein said living tissue comprising vascular endothelial

cells is transfected using means selected from the group consisting of catheter-based administration, injection-based administration, infusion-based administration, localized intravascular deliveries, liposome-based deliveries, and administrations using target-directed peptides.]

**Cancelled:** [19. The method for stimulating angiogenesis in-situ as recited by claim 14 wherein said method is practiced under in-vitro conditions.]

Respectfully submitted,

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